Evaluation of antibacterial activity of ethanolic root extract of Taraxacum Syriacum Boiss against standard and isolated...

Conference Paper · January 2015

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DOI: 10.4149/BLL_2015_008

Bratisl Lek Listy 2015; 116 (1)

41 – 46

EXPERIMENTAL STUDY

Chemical composition and hepatoprotective activity of ethanolic root extract of *Taraxacum Syriacum Boiss* against acetaminophen intoxication in rats

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Abstract: *Aim:* In the present study, the role of ethanol extract of root of *Taraxacum Syriacum Boiss* (TSBE) against hepatotoxicity caused by acetaminophen (APAP) was studied.
Methods: The chemical composition of roots of *Taraxacum Syriacum Boiss* was analyzed by SPME-GC/MS method. Hepatocellular injuries induced by acetaminophen (APAP) were assessed by liver histology, serum aminotransferase activities, antioxidant enzymes activity and lipid peroxidation in liver tissue.
Results: TSBE was observed to exhibit hepatoprotective effect as demonstrated by significant decrease in serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), and alkaline phosphatase (ALP) concentration, and by preventing liver histopathologic changes in rats with APAP hepatotoxicity. Administration of APAP, significantly increased, lactate dehydrogenase (LDH) and catalase (CAT) activity in liver tissue and pretreatment with TSBE returned these parameters to control group, moreover TSBE reduces APAP-induced hepatic Glutathione (GSH) depletion. Carvacrol (6.7 %) was the main polyphenolic compound of plant sample. Our results demonstrated hepatoprotective activity of TSBE in rat in vivo.
Conclusions: We believe that the mechanism by which the extract was able to protect the liver from the oxidative stress generated by APAP is due to its antioxidant activity. These phenolic compounds of the extract act as antioxidants and free radical scavengers and reduce or inhibit the oxidative stress induced by APAP administration *(Tab. 3, Fig. 3, Ref. 39)*. Text in PDF *www.elis.sk.*
Key words: hepatoprotective, *Taraxacum Syriacum Boiss,* acetaminophen, phenolic compounds.

Acetaminophen (paracetamol, N-acetyl-p-aminophenol; APAP) is one of the common drugs used for its analgesic and antipyretic effects (1–2). At therapeutic doses, it is believed APAP is safe and principally metabolized in liver by glucuronidation and sulfation (1, 3). In spite of the fact that APAP is safe at therapeutic doses, but it induces acute liver failure through the production of centrilobular hepatic necrosis when taken in higher doses (4–6). Previous studies have shown increases in Reactive oxygen species (ROS) production during APAP toxicity has a central role in APAP-induced damage to hepatocytes (2, 4).

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Acknowledgements: This study was supported by the grants from Razi Herbal Medicines Research Center, Lorestan University of Medical Sciences, Khorramabad, Iran.

At physiologic condition, Reactive oxygen species (ROS) when produced by cells at low and controlled levels act as second messengers (7–8). Maintaining intracellular ROS content at low physiological concentration is essential for the survival of cells, and ROS neutralizing system disorders contribute in several human diseases such as liver injury, cancer, diabetes mellitus, atherosclerosis, neurodegenerative diseases, rheumatoid arthritis, infertility, and other diseases (8–9). ROS regulates many physiological functions of cells, for example they are involved in regulation of vascular tone, responses versus infectious agents, sense of oxygen tension in the control of ventilation, regulation of cell adhesion (8). On the other hand, ROS overproduction has deleterious effects on cells by inducing oxidative stress (OS) (8). To control the level of ROS and to protect cells under OS conditions, mammalian cells contain several antioxidant agents such as alfa-tocopherol, ascorbic acid, catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (8). APAP toxicity induces antioxidant depletion and thereby deprives hepatocytes from protection by their antioxidant systems. On the other hand, a number of studies have shown that antioxidant treatment significantly protects hepatocytes against APAP toxicity (5–6). *Taraxacum Syriacum Boiss* (Family Asteraceae), commonly known as dandelion, is used in traditional Iranian herbal medicine for the treatment of jaundice, liver disorders and gallstones (10). Previous studies have shown some species of this plant through their polyphenol compounds

have beneficial effects (11–12). These compounds have antioxidant activity and this would protect the hepatocytes against overproduction of ROS by cytochrome P450 during APAP-induced hepatotoxicity. Therefore, this study was designed to investigate the effects of the ethanol extract of root of *Taraxacum Syriacum* on APAP-induced hepatotoxicity. The parameters evaluated included liver function markers in the serum and tissue, as well as on antioxidant enzyme levels and hepatic histopathology of the rat liver showing APAP-intoxication hepatotoxicity.

Materials and methods

Chemicals

The study received the approval of the Lorestan University of Medical Sciences. All chemicals were obtained from Sigma-Aldrich (USA), unless otherwise stated.

Preparation of plant material

Roots of *Taraxacum Syriacum Boiss* were collected from Khorammabad, Lorestan, Iran. It was validated by comparison with reference specimens kept at the Agriculture and Natural Resources Research Center of Lorestan, Iran. Voucher Herbarium Specimens (Collection number 12125) were deposited in the Herbarium for future references. Coarse powder from dried roots of *Taraxacum Syriacum Boiss* was extracted to exhaustion with ethanol (70 %) using a soxhlet apparatus with ethanol solvent systems. The yield of ethanol dried extract was 11.76 %. The extract was dissolved in sterilized distilled water before oral administration to the experimental animals.

Determination of composition by using

Solid Phase Microextraction-Gas Chromatography/Mass Spectrometer *(SPME-GC/MS)* method *to roots of Taraxacum Syriacum Boiss*

Composition of roots of *Taraxacum Syriacum Boiss* was determined by SPME-GC/MS method as previously described (13).

Briefly, SPME method with a polydimethylsiloxane (PDME) fiber was used in this study. The samples were placed in ultrapure water and the mixture was vigorously shaken by hand. The samples were then placed into the agitator tray and 10 min incubation time was applied for equilibration of volatiles between the headspace and sample matrix, during which the sample was heated to 60 °C. The components were collected by the fiber from the sample headspace and directly injected into a GC-MS injection port for analysis (13).

Animals and treatment

Experiments were carried out on 30 male wistar rats weighing 280–320 g. The rats were maintained under controlled conditions with temperature at 22–24 °C, relative humidity of 50–60 % and a 12 hour lighting cycle and permitted ad libitum access to water and standard lab chow. The experimental animals were divided into five groups (n = 6 per group). APAP was dissolved in 40 % polyethylene glycerol for administration.

The control group was intragastrically (i.g.) given water for seven days, and then intraperitoneally injected with isotonic 0.9 % NaCl. The APAP group served as hepatotoxicity control and was i.g. given water for seven days and then intraperitoneally intoxicated with 700 mg/kg, i.p. APAP. The TSBE 50+APAP, TSBE 100+APAP and TSBE 200+APAP groups were treated with the ethanol extracts of roots of Taraxacum Syriacum Boiss (50 mg/kg i.g., 100 mg/kg i.g., and 200 mg/kg i.g. respectively) for seven days. Six hours after the final treatment rats were intoxicated with 700 mg/kg, i.p. APAP. Twenty-four hours after the APAP administration, animals were anaesthetized and blood was collected by cardiac puncture. The liver was immediately taken out and washed with ice-cold saline and stored at –70 °C. The blood and liver samples were assessed for their biochemical and antioxidant activities, as well as histological observation.

Evaluation of liver functions

The blood samples were allowed to clot for 20–30 min. Serums were separated by centrifugation (4000 rpm) at 37 °C for 5 min and used for estimation of various biochemical parameters. The activities of serum glutamate oxaloacetate transaminase (SGOT), of serum glutamate pyruvate transaminase (SGPT) and of alkaline phosphatase (ALP) were estimated by using commercial kits (Pars Azmoon, Iran) and employing an auto-analyzer (Roche Hitachi Modular DP Systems, Mannheim, Germany).

Measurement of antioxidant enzymes activity and lipid peroxidation in liver tissue

The liver tissues were homogenized in 150 mM Tris-HCl buffered saline (pH 7.2) with a polytron homogenizer and prepared in 20 % homogenate (w/v). Superoxide dismutase (SOD) activity was assayed according to the method of Worthington (14). Absorbance was monitored at 560 nm during 5 min to measure SOD activity and the data was expressed as units/mg protein.

Catalase (CAT) activity was measured as described by Aebi (1984) (15). Absorbance was observed at 240 nm for 3 min to measure CAT activity and the data was expressed as units/mg protein.

Glutathione (GSH) content was estimated according to the method of Tietz (16). Absorbance was monitored at 412 nm and the data was expressed as nmol/mg protein.

Malondialdehyde (MDA) was measured as an index of lipid peroxidation by using the method of Satoh (17). MDA content was measured at 532 nm and data are expressed as nmol MDA per milligram of protein (nmol/mg protein).

Evaluation of LDH activity in liver tissue

The activity of Liver lactate dehydrogenase (LDH) was estimated by using commercial kits (Pars Azmoon, Iran). Absorbance was observed at 340 nm for 3 min and the data was expressed as units/mg protein.

Histological evaluation

The rat liver tissues were fixed with 10 % formalin buffer solution (pH 7.4) for 24 h and dehydrated with a sequence of ethanol solution and embedded in paraffin. The serial sections were cut 5 μm thick and stained with haematoxylin-eosin (HE), and then the extent of APAP-induced necrosis was evaluated based on morphological changes in liver sections.

Statistical analysis

One-way ANOVA analysis of variance was used for comparisons in biochemical markers followed by the Tukey´s test. Data on biochemical markers are reported as mean ± SD. Differences were considered to be statistically significant when $p < 0.05$.

Results

The compounds of roots of Taraxacum Syriacum Boiss sample by SPME-GC/MS were determined and the list of the constituents identified, in order of their elution from column, it is given in Table1. Figure 1 shows the chromatogram of the Chemical compounds of the sample. The constituents were identified by comparison of their mass spectra with those in computer library and with authentic compounds. The major components were 1, 1-dimethyldiborane (5.7 %), 1-propene, 3-ethoxy (8.1 %), 3,5-octadien-2-one (5.7 %), nonanal (5.7 %), decanal (12.4 %), nonanoic acid (5.2 %) and carvacrol (6.7 %). These compounds represent 52.2 % of the total composition of plant sample and 47.8 % of the remaining were the other 14 compounds.

Tab.1. Compounds identified in the root of Taraxacum Syriacum Boiss samples by SPME-GC/MS method.

No	Compound	Percentage	Retention Time
1	1,1-dimethyldiborane-d6	5.7	1.1
2	1-propene,3-ethoxy	8.1	1.6
3	2 octenal	2.8	8.2
4	octadecanoic acid,(2-phenyl-1,3-dioxolan-4-yl)	2.3	8.5
5	3,5-octadien-2-one,(e,e)	5.7	8.7
6	2-nonanone	2.3	9.5
7	nonanal	8.4	9.9
8	benzoic acid,2-hydroxy-,methyl ester	4.3	12.8
9	decanal	12.4	13.3
10	octadecane,6-methyl	3.3	13.6
11	d-nerolidol	3.2	14.8
12	dihydro-citronellal	4.5	15.4
13	nonanoic acid	5.2	16
14	dodecane	2.5	16.5
15	carvacrol	6.7	16.7
16	2-decanal,(e)	2.3	16.9
17	pentadecane	3.4	19.8
18	1,1,3,3-d4-trans-beta-decalone	2.4	20.1
19	geranyl acetone	3.4	21.5
20	2,5-cyclohexadien-1,4-dione,2,6-bis	4.3	21.7
21	pentadecane	2.6	23

Tab. 2. Effect of ethanolic root extract of *Taraxacum Syriacum Boiss* (TSBE) on liver function marker in acetaminophen-intoxicated rats.

	Groups	SGOT(IU/L)	SGPT(IU/L)	ALP(IU/L)
A	Control	14.7±1.2	18.2±4.5	265.7±53.6
B	APAP	26.3±6.2[a]	36.6±9.6[a]	290.1±51.8
C	TSBE 50+ APAP	25.3±5.8	38.7±11.7[a]	252.5±62.5
D	TSBE 100+ APAP	15.7±9.3	38.3±12.5[a]	203.2±37.4[b]
E	TSBE 200+ APAP	14.8±6.2[b]	24.5±11.8	188.9±40.1[b]

Rats were pretreated with TSBE (50, 100 and 200 mg/kg, i.g.) once daily for 7 consecutive days. Control rats were given saline. Six hours after the final treatment, rats were treated with acetaminophen (APAP, 700 mg/kg, i.p.). Hepatotoxicity was determined 18 h later. Values are mean ± S.D., n = 6 animals per group. APAP (TSBE solvent +APAP) [a)] Significantly different from the control group ($p < 0.05$). [b)] Significantly different compared with APAP-intoxicated group ($p < 0.05$).

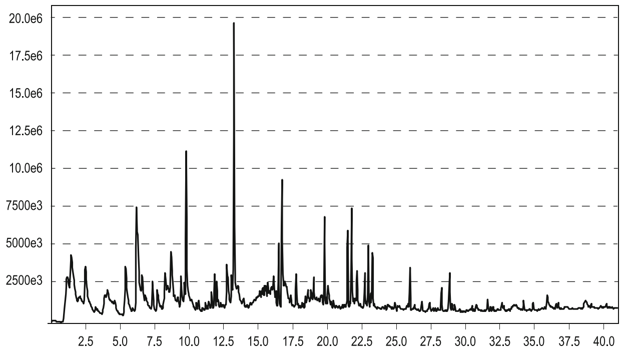


Fig. 1. Chromatogram of the root of Taraxacum Syriacum Boiss sample using the GC/MS device.

Liver function markers

The values for liver function markers are shown in Table 2. 24 hour after APAP injection, SGOT and SGPT were significantly higher ($p < 0.05$) in APAP group than Control group. While in TSBE (50, 100 and 200 mg/kg, i.g.) groups, SGOT returned to Control values. In TSBE200+APAP, SGOT activity was significantly lower ($p < 0.05$) than APAP group. SGPT in TSBE200+APAP group decreased and returned to Control group. On the other hand, ALP in TSBE 100+APAP and TSBE200+ APAP groups was significantly lower ($p < 0.05$) than APAP group and returned to the level of the control group.

Liver antioxidant enzymes and MDA levels

Data concerning liver antioxidant activity and lipid peroxidation levels are presented in Table 3. The amount of CAT was sig-

Tab. 3. Effect of ethanolic root extract of *Taraxacum Syriacum Boiss* (TSBE) on liver antioxidant enzymes-specific activities and lipid peroxidation levels in acetaminophen intoxicated rats.

	Groups	SOD (U/mg protein)	CAT (U/mg protein)	GSH (nmol/mg protein)	MDA (nmol/mg protein)
A	Control	54.1±8.7	35.5±5.6	106.5±10.8	6.70±1.4
B	APAP	45.8±8.4	49.9±7.6[b]	85.7±9.9[b]	11.1±1.8[c]
C	TSBE 50+ APAP	44.8±6.4	46.1±6.5	86.7±8.4[a]	10.7±1.5[b]
D	TSBE 100+ APAP	42.6±7.8	38.5±6.0[d]	88.8±9.3[a]	10.2±1.7[b]
E	TSBE 200+ APAP	41.3±6.3	33.5±5.3[e]	90.4±8.7[a]	9.90±1.8[a]

Rats were pretreated with TSBE (50, 100 and 200 mg/kg, i.g.) once daily for 7 consecutive days. Control rats were given saline. Six hours after the final treatment, rats were treated with acetaminophen (APAP, 700 mg/kg, i.p.). Homogenates of liver tissues were obtained from AAP-intoxicated rat at 18 h later. Values are mean ± S.D., n = 6 animals per group. APAP (TSBE solvent+APAP). [a)] Significantly different from the control group ($p < 0.05$). [b)] Significantly different from the control group ($p < 0.01$). [c)] Significantly different from the control group ($p < 0.001$). [d)] Significantly different compared with APAP-intoxicated group ($p < 0.05$). [e)] Significantly different compared with APAP-intoxicated group ($p < 0.01$).

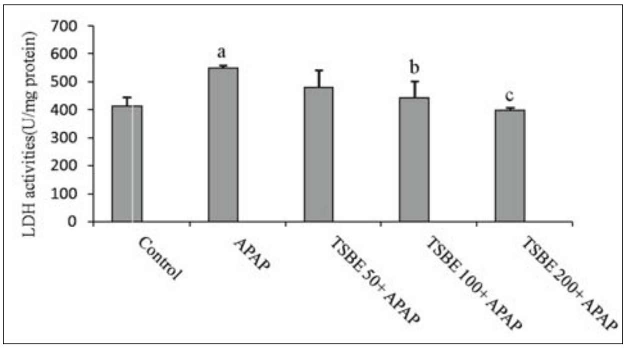


Fig. 2. Effect of ethanolic root extract of *Taraxacum Syriacum Boiss* (TSBE) on liver LDH activity in acetaminophen intoxicated rats. Rats were pretreated with TSBE (50, 100 and 200 mg/kg, i.g.) once daily for 7 consecutive days. Control rats were given saline. Six hours after the final treatment, rats were treated with acetaminophen (APAP, 700 mg/kg, i.p.). Homogenates of liver tissues were obtained from AAP-intoxicated rat at 18 h later. Values are mean ± S.D., n = 6 animals per group. APAP (TSBE solvent +APAP). a) Significantly different from the control group (p < 0.01). b) Significantly different compared with APAP-intoxicated group (p < 0.01). c) Significantly different compared with APAP-intoxicated group (p < 0.001).

nificantly increased in the APAP- intoxicated group as compared with the normal controls ($p < 0.01$) While treatments with TSBE (100 mg/kg, i.g.) ($p < 0.05$) and (200 mg/kg, i.g.) ($p < 0.01$) significantly reduced catalase activity compared with APAP group. GSH contents significantly decreased in all groups that received APAP compared to control group. APAP in all groups significantly increased MDA levels compared to Control group and TSBE was unable to prevent this adverse effect of APAP. However, there was no significant difference in the activity of SOD between all groups.

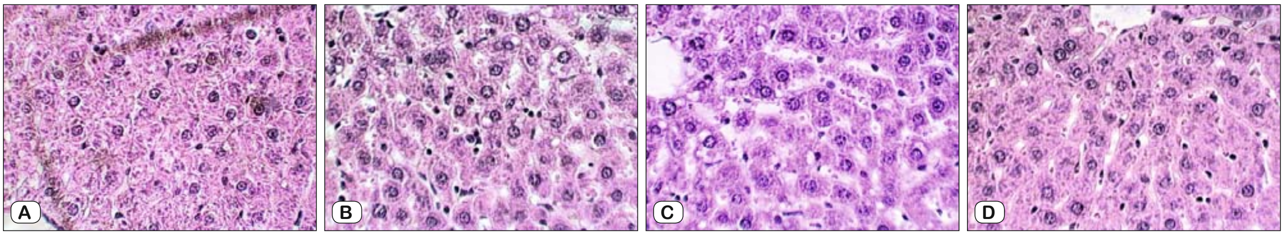
Effects of TSBE on the hepatotoxicity of APAP

LDH activities significantly increased in APAP group compared to Control group. TSBE in (100 mg/kg, i.g.) ($p < 0.01$) and (200 mg/kg, i.g.) ($p < 0.001$) significantly reduced LDH activities in comparison to APAP group (Fig. 2). Therefore TSBE reduces the cytotoxic effects of APAP on hepatocytes. Figure 3 shows the results of histopathological observations. APAP-intoxicated treatment showed histological changes such as necrosis in the centrilobular region, infiltration of the lymphocytes and Kupffer cells, ballooning degeneration and destruction of cell border (Fig. 3A). Pretreatment with TSBE 50 mg/kg did not avert the hepatic damages from APAP-induced hepatotoxicity (Fig. 3B). Pretreatment with 100 or 200 mg/kg of ethanol extract of TSBE ameliorated these histopathological damages associated with the hepatotoxicity from APAP-intoxicated treatment (Figs 3 D and E).

Discussion

In the present study, the ethanol extract of root of *Taraxacum Syriacum Boiss* was observed to exhibit hepatoprotective effect as demonstrated by significant decrease in SGOT, SGPT, and ALT concentration, and by preventing liver histopatological changes in rats with APAP induced hepatotoxicity. Administration of APAP significantly increased, LDH and CAT activity in liver tissue and pretreatment with the ethanolic extract of root of *Taraxacum Syriacum Boiss* returned these parameters to control group, suggesting that the reduction of oxidative stress in this scenario likely plays a role in the mechanism of its hepatoprotective effects. APAP is an antipyretic and analgesic drug, which is widely used to cure fever, headache and other pains, and is readily available without prescription. When taken in at toxic doses, it becomes a potent hepatotoxin, generating fulminated hepatic and renal tubular necrosis which is lethal in humans and experimental animals (18–19). The laboratory features of hepatotoxicity induced by APAP resemble other kinds of acute inflammatory liver disease with prominent increase of SGOT, SGPT and ALP levels (20).

In this present study, the serum level of hepatic enzymes SGOT, SGPT, LDH and ALP were increased and reflected the hepatocellular damage in the APAP-induced hepatotoxicity animal model. The ethanolic extract of root of *Taraxacum Syriacum Boiss*, however, could lower the SGOT, SGPT, LDH and ALP in these APAP- intoxicated animals. In addition, the examination of liver function correlated with the histopathological changes from photomicroscopy observation. Treatment with ethanolic extracts of roots of *Taraxacum Syriacum Boiss* prevented histopathological changes. Thus, these results suggested that the inhibition of liver



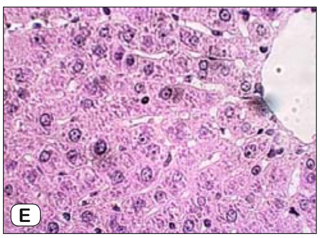


Fig. 3. Effect of ethanolic root extract of *Taraxacum Syriacum Boiss* (TSBE) on liver tissue of acetaminophen intoxicated rats. Rats were pretreated with TSBE (50, 100 and 200 mg/kg, i.g.) once daily for 7 consecutive days. Control rats were given saline. Six hours after the final treatment, rats were treated with acetaminophen (APAP, 700 mg/kg, i.p.). Liver were removed from AAP-intoxicated rat at 18 h later, then cut off and placed in 10 % formalin for histopathology assessment. APAP (TSBE solvent +APAP). (A) Liver from control group, (B) Liver from APAP group, (C) Liver from TSBE50+APAP group, (D) Liver from TSBE100+APAP group, (E) TSBE200+APAP, ×100.

function markers elevation and liver damage may participate on the protective effect of the ethanol extracts of roots of *Taraxacum Syriacum Boiss* against APAP-induced hepatotoxicity. Oxidative stress is believed to play a major role in the pathogenesis and progression of APAP-induced liver injury. It is known that the APAP metabolite (N-acetyl-p-benzoquinoneimine) rapidly reacts with glutathione which consequently exacerbates the oxidative stress (21). Reactive oxygen species such as superoxide, hydroxyl radical, iron-oxygen complexes, hydrogen peroxide and lipid peroxides are generated by several reactions. These are metabolism of triplet oxygen molecule; one electron reduction of oxygen; catalytic decomposition of hydrogen peroxide and lipid hydroperoxides by metal iron; attack of metal and/or metal-oxygen complex; irradiation of visible light and x-ray, and intake of exogenous radicals (22). These radicals react with biological molecules such as DNA, proteins and phospholipids and eventually destroy the structure of other membranes and tissues (23–25). Aerobic organs employ a battery of defense mechanisms such as antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) to prevent or mitigate oxidative tissue damage. Catalase is especially effective in dealing with the large amounts of hydrogen peroxide that is generated in the peroxisomes. Whenever the antioxidants are present, antioxidant enzyme activity and scavengers of the free radical will be induced to prevent oxidative damage (26). It is generally accepted that the initiating events in APAP-induced hepatic injury involve the formation of the toxic metabolite NAPQI by cytochrome P450-dependent enzyme systems and GSH depletion (27–30). In contrast to the toxic activation of APAP via the P450s pathway, the detoxification pathway is GSH conjugation of NAPQI, a reactive toxic metabolite of APAP. Previous studies on the mechanisms of APAP-induced hepatotoxicity have shown that GSH plays a key role in the detoxification of its reactive toxic metabolites, and that liver necrosis begins when GSH stores are markedly depleted (27, 28, 30–32). Our results show that pretreatment with the ethanol extract of the root of *Taraxacum Syriacum Boiss* significantly reduces APAP-induced hepatic GSH depletion (Tab. 3). This result is probably due to the decreased bioactivation of APAP by pretreatment with the ethanolic extract of roots of *Taraxacum Syriacum Boiss* (Tab. 3), which results in the decreased formation of NAPQI. Solid-phase microextraction (SPME) uses a fine rod (fused silica or metal) coated with a polymeric coating to extract organic compounds from their matrix and directly transfer them into the injector of a gas chromatograph for thermal desorption an analysis. It is a growing sample preparation technique, and an attractive alternative to classical extraction methods, that reduces solvent usage and exposure, disposal costs and extraction time for sample separation and concentration purposes (33–35). The compounds of the root of *Taraxacum Syriacum Boiss* sample by SPME-GC/MS were determined and carvacrol was the main polyphenolic compound of plant sample that has anti-bacterial, anti-inflammatory and anti-nociceptive effects (36, 37). The contents of the phenolic compounds have hydroxyl groups on an aromatic residue, and they exhibited antioxidant, antimutagenic, and carcinogenic activities in vitro, which were attributed to their scavenger activities against ROS (38). In the other study, *Taraxacum officinale Weber* (similar Family) leaf extract, was able to decrease thiobarbituric acid-reactive substance levels induced by APAP (p.o.), as well as prevent the decrease in sulfhydryl levels caused by APAP treatment. Furthermore, histopathological alterations, as well as the increased levels of serum aspartate and alanine aminotransferases caused by APAP, were prevented by T. officinale. In addition, T. officinale leaf extract also demonstrated antioxidant activity in vitro, as well as scavenger activity against 2,2-diphenyl-1-picrylhydrazyl and nitric oxide radicals and their results clearly demonstrated the hepatoprotective effect of T. officinale leaf extract against the toxicity induced by APAP in mice (39).

Conclusion

Our results demonstrated hepatoprotective effects of the ethanol extract of *Taraxacum Syriacum Boiss* root extract in rat in vivo. We believe that the mechanism by which the extract was able to protect the liver from the oxidative stress generated by APAP is due to its antioxidant activity. The antioxidant mechanism of the extract is probably due to its scavenger activity against several ROS attributed to the phenolic compounds. These phenolic compounds of the extract act as antioxidants and free radical scavengers and reduce or inhibit the oxidative stress induced by APAP administration.

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Received November 17, 2013.
Accepted June 9, 2014.